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(57) Abstract

Interferon-beta liquid formulations stabilized with a polyol, a non-reducing sugar or an amino acid. In particular, the formulations are stabilized with a polyol, such as mannitol. The formulations, preferably, furthermore comprise a buffer, such as acetate buffer, at a pH comprised between 3.0 and 4.0 and human albumin at a minimum quantity. The interferon-beta is preferably recombinant.

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IFN-β LIQUID FORMULATIONS

The present invention relates to liquid formulations of interferon-beta (IFN-β) stabilised with a polyol, a non-reducing sugar or an amino acid. In particular, it relates to liquid formulations containing mannitol, human albumin and acetate buffer.

Interferons (alpha, beta, gamma) are glycoproteins produced in the cells of vertebrates following induction. The most traditional inducers are virus, but also other microbial agents, other natural substances and synthetic compounds have the same behaviour.

Interferon-ß is induced in human fibroblasts, has anti-viral activity, but in the therapy of some tumoral forms other activities can be exploited together with the anti-viral, such as the anti-proliferative cellular activity and immunoregulatory activity.

Production from culture of human fibroblasts, and specifically from recombinant DNA techniques, now allows to obtain industrial quantities of interferon-beta.

It is known that proteins in the purified form are especially susceptible to degradation, even due to the normal activity of atmospheric agents. This peculiarity becomes even more evident for proteins produced according to recombinant DNA techniques.

As a direct consequence of the fact that highly purified proteins are easily subject to denaturization, it becomes desirable to obtain stable formulations which ensure the longest possible life-cycle to the product.

Stabilisation of formulations containing highly purified proteins may be carried out by the addition of one or more excipients which inhibit or delay degradation of the active principle.

Pharmaceutical compositions containing interferon-beta are well known. EP Patent application 89 245 (INTER-YEDA Ltd) describes a lyophilised composition of interferon-beta containing mannitol, human albumin and polyvinylpyrrolidone, the latter as stabilising agent. Also known are pharmaceutical liquid compositions containing other interferons.

International Patent Application WO 89/04177 (GENENTECH - Priority 03/11/87) describes liquid pharmaceutical formulations of gamma-

interferon comprising a buffer which maintains the pH within the range of 4.0-6.0, a polyhydroxylate sugar as stabiliser and a non-ionic detergent.

EP Patent Application 270 799 describes IFN-β pharmaceutical compositions in liquid form or lyophilized, which comprise, as solubilizer/stabilizer, one or more non-ionic polymeric detergents.

It is highly desirable to obtain such liquid formulations in order to avoid the reconstitution of lyophilised preparations and thus to permit ease of use.

It has now surprisingly been found that liquid pharmaceutical formulations comprising interferon-beta stabilised with a polyol, a non-reducing sugar or an amino acid in an appropriate buffer result particularly stable and maintain biological activity for a long period of time.

The main object of the present invention is to provide a liquid pharmaceutical formulation comprising interferon-beta and a polyol, a non-reducing sugar or an amino acid, as stabiliser.

Preferably the stabiliser is selected from mannitol, saccharose and glycine; more preferably, the stabiliser is mannitol.

Preferably the liquid pharmaceutical formulation comprises a buffer with a pH between 3 and 4; more preferably, acetate buffer.

Another object of this invention is to provide a process for the preparation of such liquid pharmaceutical formulation comprising the stage of dilution of IFN-β with a solution of the excipients.

Yet another object of the present invention is to provide a presentation form of the liquid pharmaceutical formulation comprising the previously mentioned formulation, hermetically sealed under sterile conditions in a container suitable for storage prior to use.

To study the stability of liquid formulations of IFN-β, various formulations were prepared diluting bulk IFN-β in different buffers at varying pH, then storing the samples at different temperatures and carrying out assays with the immunological test at set intervals of time. Once the buffer solution and the preferred pH, with which the greater stability is obtained, have been selected, then the stabilised formulations of the invention are prepared by diluting the interferon bulk solution with the buffer solution containing also the excipients. Stability of the various formulations was determined by measuring the residual activity of IFN-β at

fixed intervals of time, after storage of the solution at the temperatures of 50°C, 37°C, and 25°C.

To determine such activity, samples were assayed under immunological and biological tests.

The immunological test was carried out by using the TORAY kit (Human IFN-Beta ELISA Kit, TORAY INDUSTRIES, Inc.), following the methodology reported in the enclosed instructions.

The biological dosage was performed as described by Armstrong J.A. (1981), Cytopathic effect inhibition assay for Interferon, in Methods in Enzymology 73 381-387. This test permits the measuring of IFN-β activity by exploiting its anti-viral capacity.

Measure of activity is expressed in International Units per millilitre of solution (IU/ml) or in Mega International Units per millilitre of solution (MIU/ml). (1 MIU/ml = 1,000,000 IU/ml).

An International Unit is calculated as described in the Research Reference Reagent Note No. 35, published by the National Institute of Health, Bethesda, Maryland, in relation to the HuIFN-beta NIH Reference Reagent Gb 23-902-531 used as standard.

The measurement is reported here as percentage of residual activity of the sample of Interferon-beta in the various formulations, taking activity of the sample at time zero as equal to 100%.

Dosages were carried out in duplicate.

To assess the effect of the pH on stability of the active ingredient, different formulations of recombinant IFN-β were prepared containing 0.6 and 1 MIU/ml with various buffer solutions, i.e. acetate buffer, citrate buffer, ascorbate buffer, succinate buffer.

The formulations containing recombinant IFN-β with the buffer solutions were prepared and stored at temperatures of 50°C, 37°C and 25°C, then assayed under the immunological test at set time intervals. The formulations were prepared in such a way as to have a pH between 3.0 and 4.0 and between 5.0 and 6.0, all with buffer at a concentration of 0.01 M.

Tables 1, 2 and 3 report results of tests carried out at set intervals of time, from 1 to 42 days, at the various temperatures.

Data contained in the above-mentioned tables indicate that the formulations with a pH between 5.0 and 6.0 show an immediate loss of

titre. Formulations with pH between 3.0 and 4.0 show, however, a high stability, especially in the presence of acetate buffer.

To assess the effect of excipients on the stability of the active principle, different formulations were prepared containing 1 MIU/ml of recombinant IFN-β, using various excipients such as mannitol, saccharose or an amino acid such as glycine, and human albumin already partially contained in the interferon-beta bulk solution.

Quantities of mannitol, saccharose or glycine used were such as to obtain isotonic solutions of IFN-β.

Stability studies on these formulations were carried out by maintaining samples at 50°C, 37°C, 25°C and 4°C, and measuring residual activity at the times reported in tables 4 and 5.

Data reported in tables 4 and 5 show that degradation in the formulations containing a polyol like mannitol is much lower in respect to those formulations containing saccharose or glycine.

The formulation selected for a deeper study was the one containing mannitol in 0.01 M acetate buffer at pH 3.5, which was subjected to further tests for evaluatation of the effect on stability of the ionic force and the albumin.

Solutions of IFN-β in 0.01 M acetate buffer at pH 3.5, were prepared at different values of osmolality: 150, 300 and 400, and with different dielectric constants, with 5, 10, and 20 % propylene glycol, and samples were then stored and assayed at 50°C, 37°C and 25°C. The study shows that increase of osmolality and the propylene glycol content decreased stability of the liquid formulations of IFN-β.

Since bulk IFN-β contains albumin, it was decided to proceed to a study for evaluation of the effect of albumin on the stability of interferon-ß liquid formulations. Samples containing IFN-β (1 MIU/ml) and the acetate buffer solution at pH 3.5 were added to 1, 3, 6, and 9 mg/ml of human albumin and tested at temperatures of 50°C, 37°C and 25°C.

Results show that with the increase of albumin the stability of the samples decreased. The albumin content per sample was fixed in such a way as to have the minimum quantity compatible with that contained in the various bulks: in a formulation containing 1 MIU/ml of IFN-β, a uniform content of 0.5 mg/ml albumin is maintained.

EXAMPLES of PHARMACEUTICAL PRODUCTION

Materials : mannitol (Merck); human albumin (Boehring); 0.01 M acetate buffer (Merck); NaOH 1M (Merck).

DIN 2R glass bottles (glass type I borosilicate glass) with stoppers of Pharmagummi rubber, butylic mixture, and aluminium ring, were used as containers.

Example of preparation of r-IFN-β solution

A) SOLUTION AT 1 MIU/ML

For the preparation of a batch of 1 Lt. of finished product, the following quantities are used:

r-interferon-beta	1000 MIU
Mannitol	54.6 g
Human albumin	0.5 g-P
0.01 M pH 3.5 acetate buffer	q.s. to 1 Lt.

P = amount of human albumin present in bulk interferon.

B) SOLUTION AT 12 MIU/ML

For the preparation of a batch of 1 Lt. of finished product, the following quantities are used:

r-interferon-beta	12000 MIU
Mannitol	54.6 g
Human albumin	4.0 g-P
0.01 M pH 3.5 acetate buffer	q.s. to 1 Lt.

P = amount of human albumin present in bulk interferon.

C) SOLUTION AT 24 MIU/ML

For the preparation of a batch of 1 Lt. of finished product, the following quantities are used:

r-interferon-beta	24000 MIU
Mannitol	54.6 g
Human albumin	8.0 g-P
0.01 M pH 3.5 acetate buffer	q.s. to 1 Lt.

P = amount of human albumin present in bulk interferon.

Method of Preparation

The required quantity of mannitol and human albumin (taking into account the quantity of albumin present in the bulk) is dissolved in approximately 500 g of 0.01 M pH 3.5 acetate buffer. The pH is checked

and, if necessary, adjusted to the value of 3.5 ± 0.2 with diluted (1:2) acetic acid or with 1 M NaOH.

The solution is brought to the final weight of 1 Kg with 0.01 M pH 3.5 acetate buffer.

The required quantity of r-interferon beta is weighed in a beaker and brought to the final weight of 500 g with the solution of excipients.

In another beaker 500 g of solution of excipients is weighed. The 500 g of solution containing interferon-beta is filtered on a sterile membrane of 0.22 μm (DURAPORE) at a pressure not exceeding 1.5 atm. The sterile solution is collected in a glass erlenmeyer flask. Immediately afterwards the 500 g of excipient solution is filtered on the same membrane at a pressure of 1.5 atm and collected in the same erlenmeyer flask. The solution obtained is slowly mixed.

TABLE 1
r-INTERFERON-β

LIQUID FORMULATION : 1 MIU/BOTTLE
RESULTS IMMUNOLOGICAL DOSAGE :
CONCENTRATION (%)
STABILITY IN 0.01 M CITRATE BUFFER AT DIFFERENT pH VALUES

		50°c		37°C		25°c	
	T = 0	1 D	30 D	19 D	30 D	12 D	19 D
IFN/3	100 (890600 IU/ML)	73.0	8.6	88.5	69.9	100	97.5
IFN/4	100 (820900 IU/ML)	20.1	1.0	68.7	36.6	100	62.8
IFN/5	100 (532100 IU/ML)	ND					43.6
IFN/6	100 (179500 IU/ML)	ND					

D = day(s)
ND = non-determinable
IFN/3 = formulation in citrate buffer pH 3.0
IFN/4 = formulation in citrate buffer pH 4.0
IFN/5 = formulation in citrate buffer pH 5.0
IFN/6 = formulation in citrate buffer pH 6.0

TABLE 2
r-INTERFERON-ß

LIQUID FORMULATION : 0.6 MIU/BOTTLE
RESULTS IMMUNOLOGICAL DOSAGE :
CONCENTRATION (%)
STABILITY IN 0.01 M ACETATE BUFFER AT DIFFERENT pH VALUES

		50°c		37°C		25°c	
	T = 0	1 D	30 D	19 D	30 D	7 D	19 D
IFN/3	100 (549425 IU/ML)	72.6	48.3	97.5	100	100	100
IFN/4	100 (459600 IU/ML)	77.6	30.3	91.9	92.2		100
IFN/5	100 (52275 IU/ML)	45.0					
IFN/6	100 (25425 IU/ML)	57.2					

D = day(s)
IFN/3 = formulation in acetate buffer pH 3.0
IFN/4 = formulation in acetate buffer pH 4.0
IFN/5 = formulation in acetate buffer pH 5.0
IFN/6 = formulation in acetate buffer pH 6.0

TABLE 3

r-INTERFERON-ß

LIQUID FORMULATION : 1 MIU/BOTTLE

RESULTS IMMUNOLOGICAL DOSAGE : CONCENTRATION (%)

STABILITY IN 0.01 M ASCORBATE & SUCCINATE BUFFER AT pH 3.00 & 4.00

		50°C			37°C		25°C		
	T = 0	7 D	14 D	21 D	7 D	42 D	7 D	14 D	42 D
IFN/3/ASC	100 (1068400 IU/ML)	ND			32.4		76.5	10.5	
IFN/4/ASC	100 (1025000 IU/ML)	ND			15.6		80.6		
IFN/3/SUC	100 (980200 IU/ML)	62.9	54.8	22.1	92.7	87.8	96.0	62.5	97.2
IFN/4/SUC	100 (957600 IU/ML)	62.8	43.8	22.7	88.5	14.3		78.7	84.7

D = days

ND = non-determinable

IFN/3/ASC = formulation in ascorbic buffer pH 3.0; IFN/4/ASC = formulation in ascorbic buffer pH 4.0

IFN/3/SUC = formulation in succinate buffer pH 3.0; IFN/4/SUC = formulation in succinate buffer pH 4.0

TABLE 4
r-INTERFERON-ß

LIQUID FORMULATION : 1 MIU/BOTTLE
RESULTS BIOLOGICAL DOSAGE : CONCENTRATION (%)
STABILITY IN 0.01 M ACETATE BUFFER AT pH 3.5 with DIFFERENT EXCIPIENTS

		50°C	37°C		25°C		
	T = 0	49 D	49 D	3 M	3 M	6 M	9 M
ACE/SAC /3.5	100 (970000 IU/ML)	25.8	100	85.6	100	100	
ACE/MAN/3.5	100 (1150000 IU/ML)	67.8	100	90.4	91.3	100	85.2
ACE/GLY /3.5	100 (1200000 IU/ML)	49.2	100	75.8	90.0	98.3	

D = days
M = months
ACE/ SAC /3.5 = formulation in acetate buffer pH 3.5 + saccharose
ACE/MAN/3.5 = formulation in acetate buffer pH 3.5 + mannitol
ACE/ GLY /3.5 = formulation in acetate buffer pH 3.5 + glycine

TABLE 5

r-INTERFERON-ß

LIQUID FORMULATION : 1 MIU/BOTTLE

RESULTS IMMUNOLOGICAL DOSAGE : CONCENTRATION (%)

STABILITY USING DIFFERENT EXCIPIENTS IN 0.01 M ACETATE BUFFER AT pH 3.5

		50°C			37°C			25°C		4°C		
	T = 0	7 D	14 D	49 D	14 D	49 D	3 M	6 M	9 M	3 M	6 M	9 M
ACE/SAC /3.5	100 (1120000 IU/ML)	78.6	62.5	10.3	99.1	96.4	67.1	97.2		100	100	
ACE/MAN/3.5	100 (1070000 IU/ML)	90.6	74.8	60.3		100	100	100	100	100	100	100
ACE/GLY /3.5	100 (1220000 IU/ML)	77.0	47.5	19.4	100	91.0	93.9	96.9		87.7	100	

D = days

M = months

ACE/ SAC /3.5 = formulation in acetate buffer pH 3.5 + saccharose

ACE/MAN/3.5 = formulation in acetate buffer pH 3.5 + mannitol

ACE/ GLY /3.5 = formulation in acetate buffer pH 3.5 + glycine

CLAIMS

1. A liquid pharmaceutical formulation comprising interferon-beta and a polyol, a non-reducing sugar or an amino acid as stabilising agent.

2. A liquid pharmaceutical formulation according to claim 1, in which the stabilising agent is selected from: mannitol, saccharose and glycine.

3. A liquid pharmaceutical formulation according to claim 2, in which the stabilising agent is mannitol.

4. A liquid pharmaceutical formulation according to any of the claims from 1 to 3, in which interferon-beta is recombinant.

5. A liquid pharmaceutical formulation according to any of the claims from 1 to 4, in which interferon-beta is in a quantity between 0.6 and 1 MIU/ml.

6. A liquid pharmaceutical formulation according to any of the claims from 1 to 5, which comprises, furthermore, a buffer solution capable of maintaining the pH of the liquid formulation at a value between 3.0 and 4.0.

7. A liquid pharmaceutical formulation according to claim 6, in which the buffer solution is acetate buffer.

8. A liquid pharmaceutical formulation according to claim 6 or 7, in which the buffer solution has a concentration of 0.01 M.

9. A liquid pharmaceutical formulation according to any of the claims from 1 to 8, which also comprises human albumin.

10. A liquid pharmaceutical formulation according to any of the claims from 1 to 9, comprising 1 MIU/ml of interferon-beta, 54.6 mg/ml of

mannitol, 0.5 mg/ml of albumin in a solution of 0.01 M acetate buffer at pH 3.5.

11. Process for the preparation of a liquid pharmaceutical formulation according to any of the claims from 1 to 10, comprising the dilution of interferon-beta with a solution of excipients.

12. Presentation forms of the liquid pharmaceutical formulation according to any of the claims from 1 to 10, hermetically sealed in sterile conditions in a container appropriate for storage prior to use.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 5183746 (SHAKED ET AL), 2 February 1993 (02.02.93), column 13, line 11 - line 18, column 14, lines 22,23,29, column 15, line 19 --	1-12
X	US, A, 4465622 (NOBUHARA ET AL), 14 August 1984 (14.08.84), column 3, line 25 - line 36; column 5, line 16 - line 20, claims 5,16-19 --	1-12
X	WO, A1, 8902750 (CETUS CORPORATION), 6 April 1989 (06.04.89), claims 1-2 --	1-12

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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X	Dialog Information Services, file 351, WPIL, Dialog accession no. 003925794, WPI accession no. 84-071338/12, TORAY IND INC: "Stabilising beta-interferon having no sugar chain by adding polyol, e.g. ethylene glycol, glycerine or sugar, esp. oligo saccharide", JP 59025333, A, 840209, 8412 (Basic) -- --------	1-12

INTERNATIONAL SEARCH REPORT

Information on patent family members

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		AU-B- 659127	11/05/95
		AU-A- 1063392	19/03/92
		AU-A- 8019187	28/04/88
		CA-A- 1294215	14/01/92
		DE-D,T- 3750574	02/02/95
		EP-A,B- 0270799	15/06/88
		JP-A- 63179833	23/07/88
		NO-B,C- 175704	15/08/94
US-A- 4465622	14/08/84	CA-A- 1185178	09/04/85
		DE-A,C,C 3223087	17/02/83
		FR-A,B- 2510409	04/02/83
		GB-A,B- 2111061	29/06/83
		JP-C- 1444652	08/06/88
		JP-A- 58021691	08/02/83
		JP-B- 62054440	14/11/87
WO-A1- 8902750	06/04/89	AU-A- 2535188	18/04/89
		US-A- 5183746	02/02/93

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Washington D.C. 20231
United States of America

in its capacity as elected Office

Date of mailing (day/month/year) 09 January 1996 (09.01.96)	
International application No. PCT/EP95/01825	**Applicant's or agent's file reference**
International filing date (day/month/year) 15 May 1995 (15.05.95)	**Priority date** (day/month/year) 16 May 1994 (16.05.94)
Applicant SAMARITANI, Fabrizio et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

04 December 1995 (04.12.95)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer F. Gateau
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 730.91.11

Copy for the dud Original (for US)

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92*bis*.1 and Administrative Instructions, Section 422)

To:

VANNINI, Mario
Istituto Farmacologico Serono S.p.A.
Via Casilina, 125
I-00176 Rome
ITALIE

Date of mailing *(day/month/year)*: 16 November 1995 (16.11.95)	
Applicant's or agent's file reference	IMPORTANT NOTIFICATION
International application No.: PCT/EP95/01825	International filing date *(day/month/year)*: 15 May 1995 (15.05.95)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address

APPLIED RESEARCH SYSTEMS
ARS Holding N.V.
6 John B. Gorsiraweg
Curacao
Netherlands Antilles

State of Nationality: NL
State of Residence: NL
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address

APPLIED RESEARCH SYSTEMS
ARS Holding N.V.
14 John B. Gorsiraweg
Curacao
Netherlands Antilles

State of Nationality:
State of Residence:
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[XX] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[X] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No. (41-22) 740.14.35

Authorized officer
F. Gateau
Telephone No. (41-22) 730.91.11

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT COPY

To:

Vannini, Mario
ISTITUTO FARMACOLOGICO SERONO SPA
Via Casilina, 125
I-00176 Rome
ITALIE

Rec'd PCT/PTO NOV 15 1996

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Applicant's or agent's file reference: WO/295	Date of mailing (day/month/year): 08.08.96
	IMPORTANT NOTIFICATION

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/EP 95/01825	15/05/1995	16/05/1994

Applicant

APPLIED RESEARCH SYSTEMS ARS HOLDING N.V. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	J. Lausenmeyer Telephone No.

Form PCT/IPEA/416 (July 1992) P20473 (06/12/1995)

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
WO/295		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 95/ 01825	15/05/1995	16/05/1994

International Patent Classification (IPC) or national classification and IPC

A61K38/21

Applicant

APPLIED RESEARCH SYSTEMS ARS HOLDING N.V. et al.

COPY

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This **REPORT** consists of a total of 5 sheets, including this cover sheet.

   [X] This report is also accompanied by **ANNEXES**, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consists of a total of 21 sheets.

3. This report contains indications and corresponding pages relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [ ] Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04/12/1995	08.08.96
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	J. Ohnesorge Telephone No.

ERNATIONAL PRELIMINARY EXAMINATION REPORT

Intern. application No
PCT/EP95/01825

asis of the report

1. This report has been drawn up on the basis of (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.):

[ ] the international application as originally filed.

[x] the description, pages 1 - 11 , as originally filed,
pages , filed with the demand,
pages , filed with the letter of
pages , filed with the letter of

[x] the claims, Nos. , as originally filed,
Nos. , as amended under Article 19,
Nos. , filed with the demand,
Nos. 1 - 10 , filed with the letter of 20.05.96.
Nos. , filed with the letter of

[ ] the drawings, sheets/fig , as originally filed,
sheets/fig , filed with the demand,
sheets/fig , filed with the letter of
sheets/fig , filed with the letter of

2. The amendments have resulted in the cancellation of:

[ ] the description, pages
[ ] the claims, Nos.
[ ] the drawings, sheets/fig

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 1 - 10	YES
	Claims	NO
Inventive Step (IS)	Claims 1 - 10	YES
	Claims	NO
Industrial Applicability (IA)	Claims 1 - 10	YES
	Claims	NO

2. CITATIONS AND EXPLANATIONS

1). The subject-matter of the present application is concerned with a stable liquid pharmaceutical composition comprising interferon-beta and a polyol as a stabilising agent.

Document (1) = US-A-5 183 746 discloses stable liquid formulations comprising a recombinantly produced purified IFN-ß dissolved in an inert carrier medium comprising as a solubilizer/stabilizer an effective amount of one or more biocompatible non-ionic polymeric detergents or a combination of one or more non-ionic detergents. The composition can further comprise an additional solubilizing or stabilizing agent (i.e. SDS or glycerol, preferably one or more carbohydrate, and more preferably one or more sugars and include, for example sucrose, dextrose, dextran, mannitol, sorbitol,... The buffer is preferably selected from citrate, maleate, acetate and phosphate buffers. See in particular document (1) , column 13, lines 11 to 21, column 14, lines 22, 23, 29, column 15, line 10.

Document (3) = WO-A-89 02750 discloses a stable pharmaceutical composition of matter suitable for parenteral administration to animals or humans comprising a therapeutically effective amount of a recombinant interferon-beta protein dissolved in an inert carrier medium comprising as a solubilizer/stabilizer an effective amount of one or more biocompatible non-ionic polymeric detergents and an additional solubilizing or stabilizing agent such as glycerol. The liquid formulations can further comprise additional stabilizing agent preferably one or more sugars, such as sucrose and mannitol. Additional non-carbohydrate stabilizing agent can include human serum albumine which can be used alone or in combination with a sugar and glycine which is preferably used in combination with a carbohydrate stabilizing agent. See in particular, claims 1 and 2 and page 23, last paragraph.

Documents (1) and (3) teach the use of non-ionic surfactants. According to the present application, no surfactant is required for achieving stability.

Document (2) = US-A-4 465 622 refers to a method of purifying interferon, by adsorbing specifically onto a carrier containing acrylonitrile polymer and eluting the adsorbed interferon with an appropriate buffer. It is possible to stabilize interferon in the eluate by adding 0.005%-1% protein such as serum albumine, 1-10% sugar such as sucrose, mannitol, glucose, etc., or 0.01-1% of aminoacid such as glycin to the eluate. Examples 1 to 3 refer to interferon-beta. (see in particular column 3, line 25 to 36, column 5, lines 16-20, claims 5, 16-19 and the abstract. However, document (2) does not disclose the stabilizing pH range and no stability data are provided.

Document (4) = Dialog information services, file 351, WPIL. Dialog accession no. 003925794, WPI accession no. 84-071338/12, Toray IND INC: "Stabilising beta-interferon having no sugar chain by adding polyol, e.g. ethylene glycol, glycerine or sugar, especially oligo saccharide" & JP 59025333,A, 840209, 8412 (basic) discloses the stabilisation of beta-interferon having no sugar chain by adding polyol, e.g. ethylene glycol, glycerine or sugar, especially oligo saccharide. The document relates to a different pH : Tris-buffer (pH 8.0). It has been shown in the present application, on page 3, line 34 to page 4, line 2 and in the tables 1 and 2 that the formulations with a pH between 5.0 and 6.0 show an immediate loss of activity. The teaching of (4) teaches away from the present subject-matter.

The subject-matter of claims 1 to 10 is novel over the teaching of the cited document and involve an inventive step and fulfil therefore the requirements of Articles 33(2) and 33(3) PCT in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

CLAIMS

1. A stable, liquid pharmaceutical formulation comprising interferon-beta, a stabilising amount of a polyol, and a buffer capable of maintaining the pH of the formulation at a value between 3.0 and 4.0.

2. A liquid pharmaceutical formulation according to claim 1, wherein the polyol is mannitol.

3. A liquid pharmaceutical formulation according to any of the claims 1 or 2, in which interferon-beta is recombinant.

4. A liquid pharmaceutical formulation according to any of the preceding claims, in which interferon-beta is in a quantity between 0.6 and 1 MIU/ml.

5. A liquid pharmaceutical formulation according to any of the preceding claims, in which the buffer solution is acetate buffer.

6. A liquid pharmaceutical formulation according to claim 4, in which the buffer solution has a concentration of 0.01 M.

7. A liquid pharmaceutical formulation according to any of the claims from 1 to 6, which also comprises human albumin.

8. A liquid pharmaceutical formulation according to any of the claims from 1 to 7, comprising 1 MIU/ml of interferon-beta, 54.6 mg/ml of mannitol, 0.5 mg/ml of albumin in a solution of 0.01 M acetate buffer at pH 3.5.

9. Process for the preparation of a liquid pharmaceutical formulation according to any of the claims from 1 to 8, comprising the dilution of interferon-beta with a solution of excipients.

10. A container hermetically sealed in sterile conditions comprising the liquid pharmaceutical formulation according to any of the claims from 1 to 8 and appropriate for storage prior to use.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 95/01825	15 May 1995	16 May 1994
Applicant		
Applied Research Systems et al		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (See Box I).

2. [ ] **Unity of invention is lacking** (See Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] transcribed by this Authority.

4. With regard to the title,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the abstract,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is:
   Figure No. ---
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 95/01825

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 38/21
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, EMBASE, WPI, WPIL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 5183746 (SHAKED ET AL), 2 February 1993 (02.02.93), column 13, line 11 - line 18, column 14, lines 22,23,29, column 15, line 19 --	1-12
X	US, A, 4465622 (NOBUHARA ET AL), 14 August 1984 (14.08.84), column 3, line 25 - line 36; column 5, line 16 - line 20, claims 5,16-19 --	1-12
X	WO, A1, 8902750 (CETUS CORPORATION), 6 April 1989 (06.04.89), claims 1-2 --	1-12

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 Sept 1995	31. 10. 95
Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer PALMCRANTZ CAROLINA

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 95/01825

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Dialog Information Services, file 351, WPIL, Dialog accession no. 003925794, WPI accession no. 84-071338/12, TORAY IND INC: "Stabilising beta-interferon having no sugar chain by adding polyol, e.g. ethylene glycol, glycerine or sugar, esp. oligo saccharide", JP 59025333, A, 840209, 8412 (Basic) -- --------	1-12

SA 111571

INTERNATIONAL SEARCH REPORT

Information on patent family members

28/08/95

International application No.
PCT/EP 95/01825

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 5183746	02/02/93	AU-A- 2535188	18/04/89
		WO-A- 8902750	06/04/89
		AT-T- 111744	15/10/94
		AU-B- 659127	11/05/95
		AU-A- 1063392	19/03/92
		AU-A- 8019187	28/04/88
		CA-A- 1294215	14/01/92
		DE-D,T- 3750574	02/02/95
		EP-A,B- 0270799	15/06/88
		JP-A- 63179833	23/07/88
		NO-B,C- 175704	15/08/94
US-A- 4465622	14/08/84	CA-A- 1185178	09/04/85
		DE-A,C,C 3223087	17/02/83
		FR-A,B- 2510409	04/02/83
		GB-A,B- 2111061	29/06/83
		JP-C- 1444652	08/06/88
		JP-A- 58021691	08/02/83
		JP-B- 62054440	14/11/87
WO-A1- 8902750	06/04/89	AU-A- 2535188	18/04/89
		US-A- 5183746	02/02/93

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
WO/295		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 95/01825	15/05/1995	16/05/1994
International Patent Classification (IPC) or national classification and IPC		
A61K38/21		
Applicant		
APPLIED RESEARCH SYSTEMS ARS HOLDING N.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This **REPORT** consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by **ANNEXES**, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consists of a total of 21 sheets.

3. This report contains indications and corresponding pages relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04/12/1995	08.08.96
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	I. Obrecht Telephone No.

Intern. application No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

PCT/EP95/01825

I. Basis of the report

1. This report has been drawn up on the basis of (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.):

[ ] the international application as originally filed.

[x] the description, pages 1 - 11 , as originally filed,
pages , filed with the demand,
pages , filed with the letter of ,
pages , filed with the letter of ,

[x] the claims, Nos. , as originally filed,
Nos. , as amended under Article 19,
Nos. , filed with the demand,
Nos. 1 - 10 , filed with the letter of 20.05.96,
Nos. , filed with the letter of ,

[ ] the drawings, sheets/fig , as originally filed,
sheets/fig , filed with the demand,
sheets/fig , filed with the letter of ,
sheets/fig , filed with the letter of .

2. The amendments have resulted in the cancellation of:

[ ] the description, pages
[ ] the claims, Nos.
[ ] the drawings, sheets/fig

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 1 - 10	YES
	Claims	NO
Inventive Step (IS)	Claims 1 - 10	YES
	Claims	NO
Industrial Applicability (IA)	Claims 1 - 10	YES
	Claims	NO

2. CITATIONS AND EXPLANATIONS

1). The subject-matter of the present application is concerned with a stable liquid pharmaceutical composition comprising interferon-beta and a polyol as a stabilising agent.

Document (1) = US-A-5 183 746 discloses stable liquid formulations comprising a recombinantly produced purified IFN-ß dissolved in an inert carrier medium comprising as a solubilizer/stabilizer an effective amount of one or more biocompatible non-ionic polymeric detergents or a combination of one or more non-ionic detergents. The composition can further comprise an additional solubilizing or stabilizing agent (i.e. SDS or glycerol, preferably one or more carbohydrate, and more preferably one or more sugars and include, for example sucrose, dextrose, dextran, mannitol, sorbitol,... The buffer is preferably selected from citrate, maleate, acetate and phosphate buffers. See in particular document (1) , column 13, lines 11 to 21, column 14, lines 22, 23, 29, column 15, line 10.

Document (3) = WO-A-89 02750 discloses a stable pharmaceutical composition of matter suitable for parenteral administration to animals or humans comprising a therapeutically effective amount of a recombinant interferon-beta protein dissolved in an inert carrier medium comprising as a solubilizer/stabilizer an effective amount of one or more biocompatible non-ionic polymeric detergents and an additional solubilizing or stabilizing agent such as glycerol. The liquid formulations can further comprise additional stabilizing agent preferably one or more sugars, such as sucrose and mannitol. Additional non-carbohydrate stabilizing agent can include human serum albumine which can be used alone or in combination with a sugar and glycine which is preferably used in combination with a carbohydrate stabilizing agent. See in particular, claims 1 and 2 and page 23, last paragraph.

Documents (1) and (3) teach the use of non-ionic surfactants. According to the present application, no surfactant is required for achieving stability.

Document (2) = US-A-4 465 622 refers to a method of purifying interferon, by adsorbing specifically onto a carrier containing acrylonitrile polymer and eluting the adsorbed interferon with an appropriate buffer. It is possible to stabilize interferon in the eluate by adding 0.005%-1% protein such as serum albumine, 1-10% sugar such as sucrose, mannitol, glucose, etc., or 0.01-1% of aminoacid such as glycin to the eluate. Examples 1 to 3 refer to interferon-beta. (see in particular column 3, line 25 to 36, column 5, lines 16-20, claims 5, 16-19 and the abstract. However, document (2) does not disclose the stabilizing pH range and no stability data are provided.

Document (4) = Dialog information services, file 351, WPIL, Dialog accession no. 003925794, WPI accession no. 84-071338/12, Toray IND INC: "Stabilising beta-interferon having no sugar chain by adding polyol, e.g. ethylene glycol, glycerine or sugar, especially oligo saccharide" & JP 59025333,A, 840209, 8412 (basic) discloses the stabilisation of beta-interferon having no sugar chain by adding polyol, e.g. ethylene glycol, glycerine or sugar, especially oligo saccharide. The document relates to a different pH : Tris-buffer (pH 8.0). It has been shown in the present application, on page 3, line 34 to page 4, line 2 and in the tables 1 and 2 that the formulations with a pH between 5.0 and 6.0 show an immediate loss of activity. The teaching of (4) teaches away from the present subject-matter.

The subject-matter of claims 1 to 10 is novel over the teaching of the cited document and involve an inventive step and fulfil therefore the requirements of Articles 33(2) and 33(3) PCT in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

CLAIMS

1. A stable, liquid pharmaceutical formulation comprising interferon-beta, a stabilising amount of a polyol, and a buffer capable of maintaining the pH of the formulation at a value between 3.0 and 4.0.

2. A liquid pharmaceutical formulation according to claim 1, wherein the polyol is mannitol.

3. A liquid pharmaceutical formulation according to any of the claims 1 or 2, in which interferon-beta is recombinant.

4. A liquid pharmaceutical formulation according to any of the preceding claims, in which interferon-beta is in a quantity between 0.6 and 1 MIU/ml.

5. A liquid pharmaceutical formulation according to any of the preceding claims, in which the buffer solution is acetate buffer.

6. A liquid pharmaceutical formulation according to claim 4, in which the buffer solution has a concentration of 0.01 M.

7. A liquid pharmaceutical formulation according to any of the claims from 1 to 6, which also comprises human albumin.

8. A liquid pharmaceutical formulation according to any of the claims from 1 to 7, comprising 1 MIU/ml of interferon-beta, 54.6 mg/ml of mannitol, 0.5 mg/ml of albumin in a solution of 0.01 M acetate buffer at pH 3.5.

9. Process for the preparation of a liquid pharmaceutical formulation according to any of the claims from 1 to 8, comprising the dilution of interferon-beta with a solution of excipients.

10. A container hermetically sealed in sterile conditions comprising the liquid pharmaceutical formulation according to any of the claims from 1 to 8 and appropriate for storage prior to use.